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ORIGINAL ARTICLE

Low incidence of IgA isotype of HLA antibodies in alloantigen exposed individuals

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Human leukocyte antigen (HLA) antibodies are induced by pregnancy, transfusion, or transplantation. Serum from transplant recipients is regularly screened for IgG HLA antibodies because of their clinical relevance for transplant outcome. While other isotypes of HLA antibodies, such as IgA may also contribute to the alloimmune response, validated detection assays for IgA HLA antibody detection are lacking. Therefore, we modified the commonly used luminex screening assay for IgG HLA antibody detection (IgG-LMX) into an IgA HLA antibody screening assay (IgA-LMX). Optimization and validation was performed with IgG, IgA1, and IgA2 isotype variants of HLA-specific human recombinant monoclonal antibodies (mAbs). Reactivity patterns of IgA1 and IgA2 isotype HLA-specific mAbs in IgA-LMX were identical to those of the IgG isotype. Cross-reactivity with IgG and IgM antibodies and nonspecific binding to the beads were excluded. Further assay validation showed the absence of IgA HLA antibodies in serum from individuals without alloantigen exposure (n = 18). When the IgA-LMX assay was applied to sera from 289 individuals with known alloantigen exposure through pregnancy (n = 91) or kidney transplantation (n = 198), IgA HLA antibodies were detected in 3.5% of individuals; eight patients on the kidney retransplant waitlist and two women immunized through pregnancy. The majority (90%) of IgA HLA antibodies were directed against HLA class II and were always present in conjunction with IgG HLA antibodies. Results of this study show that this validated IgA-LMX method can serve as a screening assay for IgA HLA

Abbreviations: ABMR, antibody-mediated rejection; DSA, donor-specific antibody; ELISA, enzyme-linked immunosorbent assay; HLA, human leukocyte antigen; Ig, immunoglobulin; LMX, HLA antibody screening assay by luminex; mAb, monoclonal antibody; MFI, mean fluorescence intensity; PBS, phosphate-buffered saline; SAB, single antigen bead.

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antibodies and that the incidence of IgA HLA antibodies in alloantigen exposed individuals is low.

KEYWORDS

HLA antibody screening, IgA antibody, luminex, pregnancy, transplantation

1 | INTRODUCTION

The presence of IgG isotype of antibodies directed to mismatched donor human leukocyte antigen (HLA) in patients before and after kidney transplantation is considered to be a risk factor for the development of antibody-mediated rejection (ABMR) and inferior allograft survival.[1-3] However, not all patients with IgG donor specific antibodies (DSA) experience poor graft survival.[4-6] In addition, ABMR may develop in the absence of IgG DSA in some cases.[2,7-9] Explanation for these phenomena may lay in the contribution of other isotypes of HLA antibodies, such as IgA, to the alloimmune response. This isotype of HLA antibodies is not routinely determined in clinical practice. While some studies have reported superior graft survival in kidney transplant recipients with IgA HLA antibodies,[10-12] other studies have indicated IgA HLA antibodies as a contributor to allograft rejection.[13-17]

In earlier studies, enzyme-linked immunosorbent assay (ELISA)[11,13,14] and flow cytometry[16,17] methods were used for IgA HLA antibody detection whereas in recent studies, the much more sensitive luminex technology was utilized.[15,18-20] Importantly, in the later studies IgA-specific HLA antibody detection was achieved by replacing the standard luminex polyclonal IgG detection antibody with monoclonal IgA1/2 detection antibodies, with validation performed by using a chimeric mouse/human HLA-class II specific IgA monoclonal antibody.[15,18,20-22] Using bead-based luminex technology, Arnold et al reported the presence of IgA HLA antibodies in up to 31% of serum samples from solid organ repeat transplant waitlist patients whereas Heinemann et al found IgA HLA antibodies in 4% of eluates from explanted kidneys, indicating that the presence of IgA isotype of HLA antibodies may affect allograft outcome.[20,22]

Within the Eurotransplant region, around 20% of the patients on the kidney transplant waitlist are immunized and around 5% highly immunized as a result of previous exposure to allogeneic HLA.[23] Considering that, we aimed to design a screening assay allowing us to determine the presence of HLA-specific antibodies of the IgA isotype. To this aim, we modified and optimized the commonly used luminex bead-based screening assay for a reliable IgA HLA antibody detection. We validated the assay with IgA isotype of recombinant HLA-specific human mAbs, resulting in a luminex bead-based IgA HLA antibody screening assay with comparable sensitivity to the standard IgG HLA antibody screening assay. In addition, in the same way we developed and validated IgA specific single antigen bead (SAB) assay that enabled further analysis of IgA HLA antibody screening results. These methods were subsequently used to determine the frequency of IgA isotype of HLA-specific antibodies in the presence and absence of IgG HLA antibodies in serum samples from individuals who had been alloantigen exposed through transplantation or pregnancy.

2 | MATERIALS AND METHODS

2.1 | Reagents

For IgA HLA antibody detection, the following detection antibodies were used: mouse anti-human IgA1 (clone: B3506B4), mouse anti-human IgA2 (clone: A9604D2), goat anti-human pan IgA-PE, heavy chain-specific (all from Southern Biotech, Birmingham, AL), and mouse anti-human IgA2 (clone: IS11-21E11, MACS Miltenyi Biotec). For optimization and validation of the IgA-LMX and IgA-SAB assay, the following in-house developed HLA-specific mAbs were used: human recombinant -IgA1 and -IgA2 mAbs derived from the previously described MUS4H4-IgG1, recognizing HLA-Bw4/A24/A32/A25 and B cell hybridoma derived human mAbs (IgG, SN607D8 recognizing A2/A28 and IgM blend, which is a mixture of 10 different human mAbs of the IgM isotype together recognizing the complete panel of HLA-class I coated beads included in the SAB panel).[24,25]

2.2 | Serum samples

A total of 307 serum samples were obtained with informed consent under guidelines issued by the medical ethics committee of Leiden University Medical Center (Leiden, the Netherlands).

Serum samples were collected from individuals who had never been exposed to alloantigens (n = 18) and

TABLE 1 Characteristics of the study population

Study population group	IgG HLA Ab positive	IgG HLA Ab negative
Kidney transplant waitlist patients with a history of tx (n = 84)		
n	41	43
F/M	18/23	21/22
Number of previous tx, median (range days)	2 (1–5)	1 (1–2)
Time from last tx failure to sampling, median (range days)	917 (0–3940)	53 (2–2163)
Women with a history of pregnancy (n = 91)		
n	55	36
Number of previous pregnancies, median (range days)	1 (1–2)	2 (1–7)
Time from last delivery to sampling, median (range days)	457 (3–8602)	1 (0–18)
Patients with functioning grafts (n = 109)		
n	25	84
F/M	6/19	14/70
Time from tx to post-tx sampling, median (range years)	5 (1–11)	1 (1–14)
Patients with the biopsy-proven early ABMR (n = 5)		
F/M	0/0	5/0
Time from transplantation to post-tx sampling/ ABMR diagnosis, median (range days)	—	7 (6–13)

Abbreviations: Ab, antibody; ABMR, antibody-mediated rejection; F, female; HLA, human leukocyte antigen; M, male; n, number; tx, transplantation.

from individuals who had been exposed to alloantigens (n = 289) through kidney transplantation (n = 198) or pregnancy (n = 91).

Samples from kidney transplant recipients included: (a) 84 samples from selected kidney waitlist patients with a history of at least one previous transplantation, (b) 109 post-transplant serum samples from transplant recipients with functioning graft at the time of sampling, and (c) 5 post-transplant samples from patients with biopsy-proven early ABMR collected within 6–13 days after transplantation, in the absence of detectable IgG isotype of HLA antibodies.

Samples from women exposed to alloantigens through pregnancy had either IgG HLA antibodies (n = 55) or no detectable serum HLA antibodies of IgG isotype (n = 36), based on the presence of an uninterrupted pregnancy for at least 37 weeks and a minimum of one HLA-A, -B, or -DR mismatch between the mother and the child and negative IgG-LMX result (Table 1).

2.3 | Development of IgA HLA antibody screening and single antigen bead assays

For the development of the IgA HLA screening assay, Lifecodes Life Screen Deluxe Kit (Immucor Transplant Diagnostics, Stamford, CT) was used. Modification of the assay was performed by replacement of standard IgG-PE secondary antibody (Immucor Transplant Diagnostics, Stamford, CT) with the goat anti-human pan IgA-PE, selected upon the specificity and sensitivity tests conducted on human HLA-specific monoclonal antibodies (MUS4H4-IgA1 and -IgA2). In the same way, the IgA SAB assay with Lifecodes LSA Class I Kit, (Lifecodes, Immucor, Transplant Diagnostics, Stamford, CT) was modified and then further used to test cross-reactivity and nonspecific binding of the selected IgA-PE detection antibody on IgG and IgM isotype of HLA mAbs and PBS sample. Using LMX and SAB assays, IgA detection was compared with standard IgG detection on human HLA-specific monoclonal antibodies with exactly the same specificity and affinity but different isotypes (MUS4H4-IgG, -IgA1, and -IgA2). Validation of the IgA HLA screening assay was further performed on serum samples from alloantigen nonexposed individuals and exposed individuals. To test the detectability of the IgA HLA antibodies in serum environment, serum from alloantigen nonexposed individual spiked with IgA1 and IgG1 mAbs recognizing the same (MUS4H4) or different epitopes (MUS4H4 and SN607D8) was analyzed with the modified IgA-SAB assay.

2.4 | Detection of HLA antibodies in serum samples

All serum samples were screened for the presence of IgG and IgA isotype of HLA antibodies using Lifecodes Life Screen Deluxe Kit (Immucor) (IgG-LMX and IgA-LMX) and further tested with Lifecodes LSA Class I and Class II Kits (Immucor) for IgG and/or IgA following a previously described protocols,[26] when IgA-LMX positive or necessary. Briefly, serum samples were incubated either with a mixture of class I (7 groups) and class II (5 groups) beads in LMX or single antigen coated class I or class II beads in SAB assays for 30 min and upon washing, IgG or IgA isotype of antibodies bound to the beads were detected using a PE-conjugated goat-anti-human IgG or IgA detection antibody, respectively. All serum samples

were EDTA treated prior to luminex analysis for the IgG HLA antibody identification. Samples were acquired on a luminex flow analyzer (LABScan 100) and data were analyzed using the Match IT! Antibody software version 1.3.0 (Lifecodes, Immucor). IgG-LMX and IgG-SAB results were assigned positive or negative according to the software assignment and the lot-specific cut-off. The cut-off MFI value for IgA-LMX screened serum samples was determined on basis of the reactivity of the 18 sera from non-alloantigen exposed individuals using the Tukey formula: (third quartile + [3× interquartile range]) and subsequently determining positivity in the IgA-SAB assay on randomly selected samples having raw MFI values ranging up to 500 MFI in the IgA-LMX assay. For the IgA-SAB assay cut-off for positivity was not defined. Instead, the highest-ranked HLA alleles identified in IgA-SAB assays were related to previous HLA immunization of tested individuals.

2.5 | Statistical analysis

The Kruskal–Wallis test was used for unpaired analysis and the Mann–Whitney U tests with Bonferroni

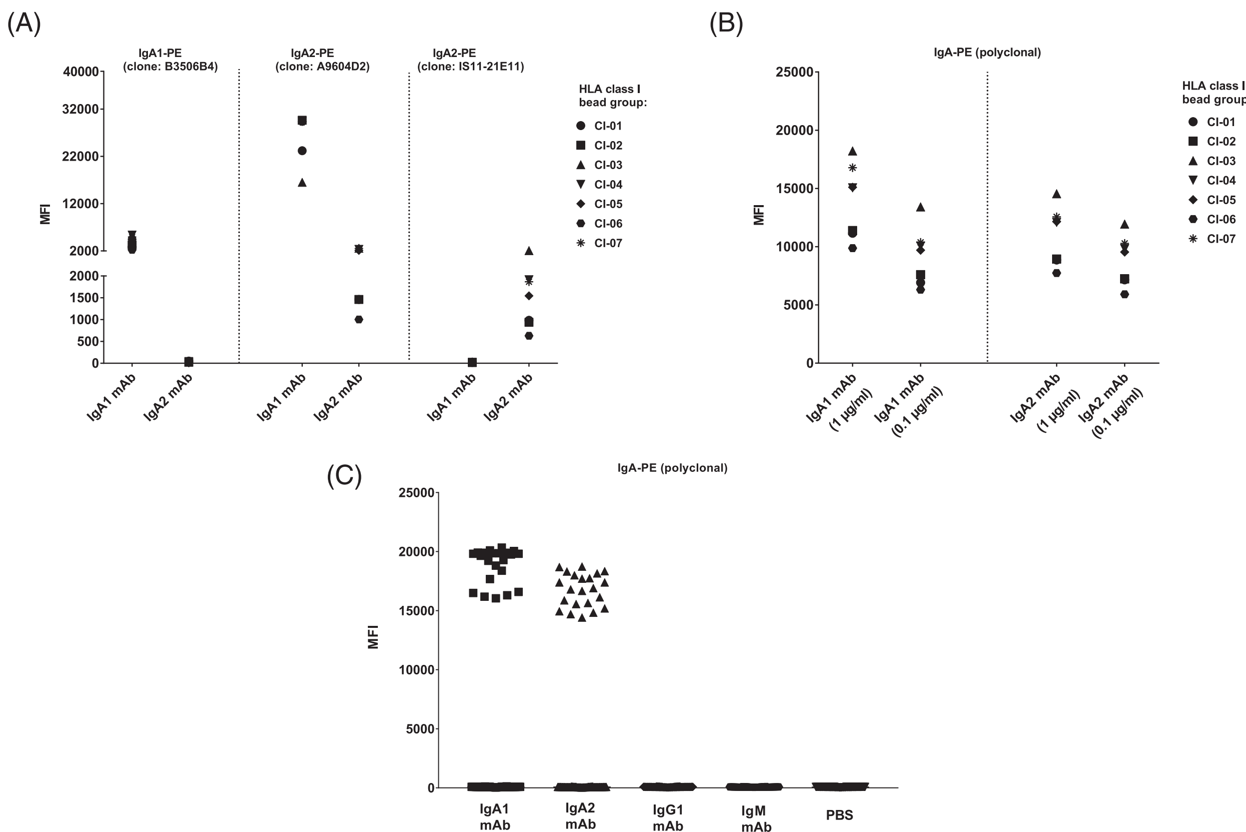


FIGURE 1 Luminex screening assay for IgA human leukocyte antigen (HLA) antibody detection using monoclonal and polyclonal IgA detection antibodies. (A) Monoclonal IgA antibody detection (IgA1-PE, clone: B3506B4 and IgA2-PE, clone: A9604D2) applied on human recombinant monoclonal IgA1 and IgA2 HLA class I antibodies (1 μg/ml) showed cross-reactivity between IgA1 antibody sample and IgA2-PE (clone: A9604D2) detection antibody (median MFI: 29385, range: 16520–29,657) (middle panel). Cross-reactivity was not observed when detection antibody IgA2-PE (clone: IS11-21E11) was used on the human recombinant monoclonal IgA1 antibody sample (median MFI: 21, range: 18–31) (right panel) but the MFI detection signal when applied on IgA2 antibody sample (median MFI: 1544, range: 630–2068) was lower than detection signal from IgA1-PE detection on IgA1 antibody sample (median MFI: 4376, range: 2234–5400) (left panel). (B) Compared with monoclonal antibody detection, polyclonal antibody detection gave higher IgA detection signal when applied on IgA1 (median MFI: 15049, range: 9874–18,220 (1 μg/ml)) and IgA2 (median MFI: 12059, range: 7741–14,546 (1 μg/ml)) antibody samples. (C) Specificity of the polyclonal IgA detection antibody was confirmed in the IgA-SAB assay which showed very high MFI values for all 22 MUS4H4 specificities when IgA-PE was applied on MUS4H4-IgA1 (MFI range: 29–20,336) and MUS4H4-IgA2 (MFI range: 33–18,725) mAb samples. Cross-reactivity was not observed when IgA-PE was applied on MUS4H4-IgG1 mAb (MFI range: 35–116) as well as IgM mAb mixture (MFI range: 41–115). Nonspecific binding to the beads was excluded by testing the detection on PBS sample (MFI range: 34–100)

correction were used for unpaired multiple analyses. Statistical level of significance was defined as $p < 0.05$, and analyses were performed with the GraphPad Prism, version 7.02 (GraphPad Software, La Jolla, CA).

3 | RESULTS

3.1 | Selection of the detection antibody for IgA luminex bead-based assays

In order to modify the standard luminex bead-based assay for IgA HLA antibody detection, replacement of standard IgG-PE secondary antibody with an IgA-specific detection antibody was necessary. We first tested the performance of previously published IgA-specific mouse anti-human IgA1 (clone: B3506B4) and IgA2 (clone: A9604D2) monoclonal antibodies (mAbs),[15,20] as well as mouse anti-human IgA2 (clone: IS11-21E11). HLA-specific mAbs were used to test the specificity and sensitivity of these monoclonal IgA detection antibodies. Since only HLA class-I specific mAbs were tested, detection signals were obtained exclusively from HLA class I bead groups, as expected. Still, because of cross-reactivity of clone A9604D2 and low MFI values obtained when using clone B3506B4 or clone IS11-21E11, these mAbs were not considered to be appropriate reagents for our purpose (Figure 1(A)). Therefore, we decided to test the specificity and sensitivity of a polyclonal IgA detection antibody conjugated to PE on IgA1 and IgA2 HLA-specific mAbs.

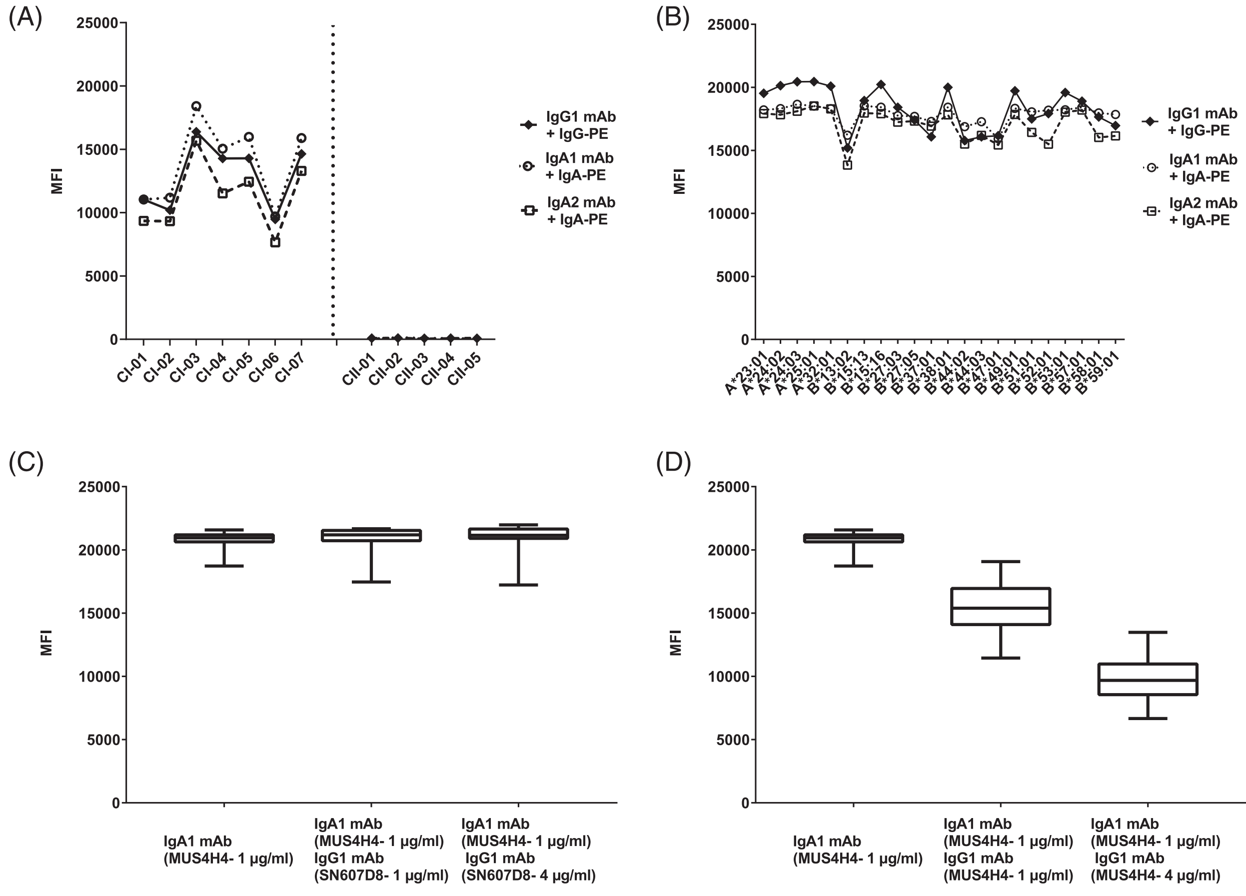


FIGURE 2 Comparable sensitivity between IgA and IgG bead-based assays. (A) IgA screening results for MUS4H4-IgG and -IgA human recombinant human leukocyte antigen (HLA) class I monoclonal antibodies prepared in the equimolar solutions (6.3 nM ~1 µg/ml) showed comparable detectability of IgA (IgA1 mAb median MFI: 15044, range: 9693–18,409; IgA2 mAb median MFI: 1152, range: 7655–15,670) and IgG (median MFI: 14275, range: 9474–16,384) antibodies. Thereby, no HLA class II reactivity was detected.
(B) Detectability of IgA and IgG was further confirmed in single antigen bead assay where all 22 MUS4H4 specificities were detected. (C) In serum from a nonimmunized individual spiked with IgA HLA antibody (1 µg/ml) and IgG HLA antibody recognizing a different epitope (1 and 4 µg/ml), detectability of IgA HLA antibody (1 µg/ml) (median MFI: 20954, range: 16044–20,336) was not influenced by the presence of the IgG isotype in 1:1 ratio IgA and IgG (median MFI: 15386, range: 11449–19,075) or 1:4 ratio IgA and IgG mAbs (median MFI: 9683, range: 6669–13,476). (D) When serum sample was spiked with IgG HLA antibody (1 and 4 µg/ml) recognizing the same epitope as IgA HLA antibody, IgA (1 µg/ml) (median MFI: 20954, range: 18722–21,576) gave lower signal in 1:1 ratio IgA and IgG (median MFI: 21187, range: 17464–21,668) and 1:4 ratio IgA and IgG mAbs solutions (median MFI: 21157, range: 17226–21,979)

This polyclonal detection antibody provided a high signal in the IgA-LMX assay (Figure 1(B)). Specificity of the polyclonal IgA detection antibody was confirmed using the IgA-SAB assay, which showed no cross-reactivity when IgA-PE detection was used on IgG or IgM isotype of mAbs. Nonspecific binding of the IgA-PE to the beads was excluded by using PBS as the test sample (Figure 1 (C)). Subsequent experiments were performed using this polyclonal IgA-PE detection antibody.

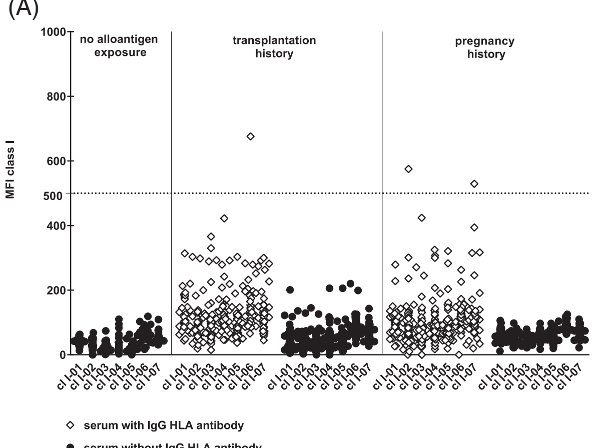
3.2 | Comparison of the sensitivity of polyclonal IgA detection versus IgG detection

Next, the sensitivity and specificity of the polyclonal IgA detection antibody was compared with the standard IgG detection by testing IgA1, IgA2, and IgG1 isotype variants of the same human mAb (MUS4H4) using LMX and SAB tests. When the polyclonal IgA detection antibody was used to detect IgA1 and IgA2 mAbs, MFI values obtained in the LMX assays were comparable to IgG detection (Figure 2(A)). In IgA-SAB assays, all 22 HLA-class I specificities identified by IgG1 MUS4H4 mAb were also detected by IgA1 MUS4H4 (MFI range: 15,926–18,651) and IgA2 MUS4H4 (MFI range: 13,837–18,529) mAbs with similar MFI values to IgG detection (MFI range: 15,189–20,453) (Figure 2(B)).

Spiking experiments were performed to test the detectability of the IgA HLA mAbs in a serum matrix from a nonsensitized individual, in the presence of IgG HLA mAbs recognizing a different, or the same epitope. In serum spiked with IgA1 (MUS4H4) and IgG1 (SN607D8) mAbs in 1:1 and 1:4 ratio, detectability of IgA1 was not influenced by the presence of an IgG mAb recognizing a different epitope (Figure 2(C)). When IgA1 and IgG1 (both MUS4H4) mAbs were mixed in a serum sample, IgA1 mAb was still detectable, albeit with relatively lower MFI values in 1:1 (median: 15,386) and 1:4 (median: 9683) IgA1:IgG1 mAb ratios, compared with serum spiked solely with IgA1 mAb (median: 20,954) (Figure 2(D)) suggesting competition between IgA and IgG isotypes when the same epitope is recognized.

3.3 | IgA luminex screening assay cut-off determination

To establish a cut-off for positive responses in IgA-LMX assay, we used a two-step approach. We first screened 18 serum samples from alloantigen nonexposed individuals and defined the outlier values. Accordingly, a cut-off of 250 raw MFI was calculated. However, in randomly selected samples having raw MFI values ranging up to 500 in IgA-LMX assay, we did not detect any IgA isotype of HLA antibodies using IgA-SAB assay (data not shown). Therefore, samples with raw MFI values >500



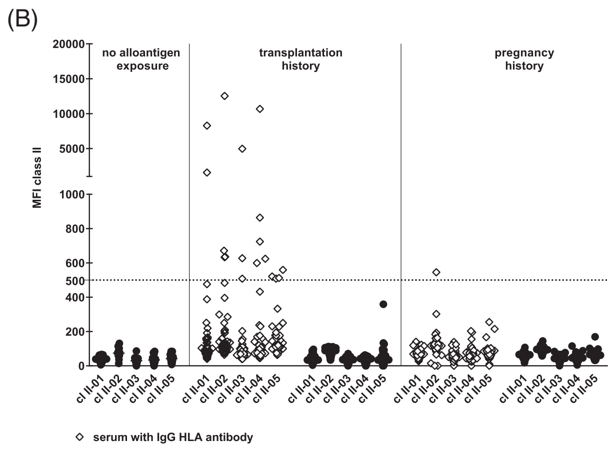


FIGURE 3 IgA human leukocyte antigen (HLA) class I (A) and class II (B) screening results for the samples from individuals with a different history of alloantigen exposure. Every individual is represented with seven dots (bead groups) for HLA class I and five dots (bead groups) for HLA class II. The arbitrary cut-off of 500 MFI (represented with dotted line) for any of class I and/or class II bead groups was used to assign samples as IgA-LMX positive. IgA HLA antibodies were detected mostly against HLA class II in patients with a history of previous transplantation. When IgA HLA antibody signal was compared between the groups with a different history of alloantigen exposure, for both LMX class I and class II bead groups, a significant difference was observed (Kruskal–Wallis test, $p < 0.0001$). In addition, comparison of MFI signals obtained for IgA HLA antibody within the groups of samples with a history of transplantation and pregnancy (with and without IgG antibody), performed for each LMX class I and class II bead group showed existing significant difference (Mann–Whitney U tests with Bonferroni correction, all $p < 0.05$)

TABLE 2 Overview of the IgA HLA antibody positive screening sample result. Positive screening results for IgA HLA antibodies included two samples from women immunized through pregnancy and eight from transplant recipients. IgA HLA antibodies were directed against only class I ($n = 1$), only class II ($n = 8$) and both class I and II ($n = 1$) HLA antigens. The highest ranked antibodies were DSA in six samples, non-DSA in one sample and nonspecified (typing not available) in four samples

Sample	Immunization history	IgA-LMX class I	IgA-LMX class II	Self HLA	Highest ranked specificity (IgA-SAB)	Raw MFI IgA-SAB	Status of the highest ranked specificity
1	Transplantation	Pos	Pos	A*01:01, A*02:01, B*13:02, B*37:01, C*06:02, DRB1*07:01, DRB1*10:01, DQB1*02:01, DQB1*05:01	B*08:01/ DRB1*11:01	1602/877	cl I DSA/cl II non-DSA
2	Transplantation	—	Pos	DRB1*07, DRB1*12, DQB1*03:01, DQB1*03:03, DQA1*02:01, DQA1*05:05	DRB1*04:05	9178	cl II DSA
3	Transplantation	—	Pos	A*01:01 A*11:01, B*08:01, B*44:02, C*05:01, C*07:01, DRB1*03:01, DRB1*12:01, DRB3, DQB1*02:01, DQB1*03:01	DRB4*01:01	661	cl II DSA
4	Transplantation	—	Pos	A*02:01, A*03:01, B*07:02, B*27:05, C*02:02, C*07:02, DRB1*13:02, DRB1*15:01, DQB1*06:02, DQB1*06:04, DQA1*01:02, DPB1*03:01, DPA1*01:03, DPB1*04:01	DQB1*03:01-DQA1*05:01	405	cl II DSA
5	Transplantation	—	Pos	A*31:01, A*32:01, B*15:18, B*40:01, C*03:04, C*07:04, DRB1*04:04, DRB1*04:04, DQB1*03:01, DQB1*03:02	DQB1*05:01-DQA1*01:02	255	cl II DSA
6	Transplantation	—	Pos	A*03:01, A*33:01, B*15:03, C*02:10, DRB1*10:01, DRB1*11:01, DQB1*03:01, DQB1*05:01	DPB1*28:01-DPA1*02:02	230	NS
7	Transplantation	—	Pos	A*02:01, A*24:02, B*08:01, B*27:05, C*01:02, C*07:01, DRB1*03:01, DRB1*07:01, DQB1*02:01, DQB1*02:01	DPB1*04:01-DPA1*02:01	1479	NS

(Continues)

TABLE 2 (Continued)

Sample	Immunization history	IgA-LMX class I	IgA-LMX class II	Self HLA	Highest ranked specificity (IgA-SAB)	Raw MFI IgA-SAB	Status of the highest ranked specificity
8	Transplantation	—	Pos	A*01, A*29, B*08, B*40, C*03, C*07, DRB1*03, DRB1*03, DRB3, DQB1*02, DQB1*02, DPB1*02:01, DPB1*04:02	DPB1*04:01-DPA1*02:01	1666	NS
9	Pregnancy	Pos	—	A1, A3, B7, Bw6, DR2, DQ1,	A*32:01	1145	cl I DSA
10	Pregnancy	—	Pos	DRB1*04:01, DRB1*12:01, DRB3*02:02, DRB4, DQB1*03:01, DQB1*03:02, DQA1*03, DQA1*05:01/05:03, DPB1*04:01, DPB1*04:02	DRB1*01:01	244	NS

Abbreviations: cl I, HLA class I; cl II, HLA class II; DSA, donor specific antibody; MFI, mean fluorescence intensity; non-DSA, non-donor specific antibody; NS, not specified (typing not available); Pos, positive.

for any of the IgA-LMX class I and/or class II bead groups were considered positive throughout the study.

3.4 | Frequency of IgA HLA antibodies in alloantigen exposed individuals

To determine the frequency of occurrence of IgA HLA antibodies related to a route of alloantigen exposure, 175 selected samples from kidney transplant waitlist patients with a history of transplantation (n = 84), and from healthy women immunized through pregnancy (n = 91) were analyzed. In the serum from repeat transplant candidates previously screened for the presence of IgG HLA antibody (43 IgG− and 41 IgG+), IgA HLA antibodies were detected exclusively in IgG HLA antibody positive samples (n = 8). IgA HLA antibodies were directed against HLA class II in seven patients and against both HLA class I and II in one patient. Among samples from women with a history of pregnancy (n = 91), grouped based on the IgG HLA antibody status (36 IgG− and 55 IgG+), IgA HLA antibodies were observed in two individuals, again accompanied by IgG antibodies. In summary, of 175 individuals with a history of alloantigen exposure, 10 (5.7%) showed detectable IgA HLA antibodies (Figure 3), as compared with 96 (54.8%) with detectable IgG HLA antibodies.

Considering that IgA HLA antibodies might have a beneficial effect on the graft survival, we also screened post-transplant sera from 109 kidney transplant patients with functioning grafts for IgA HLA antibodies (median time after transplantation 1 year). No IgA HLA antibodies were detected in this group of patients regardless of their IgG HLA antibody status (84 IgG− and 25 IgG+). Since IgA HLA antibodies might also have a role in ABMR, we screened post-transplant serum samples from five patients at time of ABMR in the absence of IgG HLA antibodies but also here did not detect any IgA HLA antibodies.

When IgA-LMX positive samples (n = 10) were further tested using the IgA-SAB assay, the highest ranked HLA alleles recognized had a median MFI of 877 (range: 230–9178) while MFI values for self-HLA ranged between 16 and 178. The highest ranked specificities in IgA-SAB assays were defined as DSA in six (MFI range: 255–9178) and non-DSA in one (MFI: 877) sample whereas in four samples (MFI range: 230–1666) the donor-specificity could not be determined because of missing HLA typing data of previous immunizations (Table 2).

4 | DISCUSSION

The clinical relevance of IgA HLA antibodies in transplantation is still unclear. This is partially because of the lack of sensitive and reliable IgA HLA antibody screening

assays that can be used routinely as part of the screening protocol in patients awaiting transplantation as well as after transplantation. Previous studies on the presence of IgA HLA antibodies in transplant recipients were performed with luminex technology on basis of monoclonal IgA detection matrix.[15,18-20,22] In our validation experiments, the previously described IgA2-specific monoclonal detection antibody showed cross-reactivity rendering this a suboptimal reagent for our purpose. Furthermore, the monoclonal reagents resulted in a significantly lower signal when compared with polyclonal reagents.[27] In the current study, we optimized a luminex bead-based IgA HLA antibody screening assay that utilizes polyclonal IgA-PE as a detection agent. Availability of IgA and IgG isotypes of the same HLA-specific human recombinant monoclonal antibodies enabled us to validate the sensitivity and specificity of the assay.

IgA antibody screening using this validated assay showed the presence of IgA HLA antibodies in about 3.5% of individuals exposed to alloantigens, which is approximately 10 times lower than the 31% reported in previous studies using luminex detection by Arnold et al[18,20] One of the reasons for this difference could be the discrepancy in the composition of the study cohorts. While Arnold et al used samples from waitlist transplant recipients, in our study 31.5% of samples belong to women exposed to alloantigens through pregnancy. It is known that pregnancy induced HLA antibodies may decrease or completely disappear over time.[28] In addition, 37.8% of study samples were from transplanted patients with functioning grafts while taking immunosuppressive medication which may affect the formation of antibodies. When only repeat transplant waitlist patients (n = 84) were analyzed, the frequency of IgA HLA antibody was still low (10%).

Alternatively, existing discrepancy in frequency may be attributed to a different definition of positivity and/or utilization of luminex bead-based kits from different vendors. The IgA HLA antibody positivity of 31% published by Arnold et al was based on IgA HLA antibody screening assay results assigned positive when the ratio between the patient sample and negative control was ≥2. In our study, for the positivity of IgA HLA antibody we used an arbitrary cut-off value for any of the class I and/or class II LMX bead groups set at raw 500 MFI. In order to test the influence of the cut-off value, we lowered our IgA-LMX threshold to raw 250 MFI, resulting in an IgA HLA antibody frequency of 19% among our samples. However, the IgA-SAB results for samples ranging 250–500 MFI in IgA-LMX, could not be explained based on the transplantation history of those patients.

In all previous studies investigating the presence of IgA HLA antibodies by luminex, One Lambda kits were used, whereas we used Lifecodes, Immucor kits. As known from the IgG HLA analyses, the two vendors differ in sensitivity, specificity, antigen panel composition, and level of denatured antigens.[29-32] In the present study, the IgA-LMX and IgA-SAB results showed sometimes a discrepant reactivity, what is in line with what was previously reported for the detection of IgG HLA antibodies.[33]

The low incidence of IgA HLA antibodies may be caused by the competition for the binding sites on HLA molecules, existing between IgG and IgA isotype of HLA antibodies, since IgG serum concentrations are significantly higher than those of IgA.[34] Our spiking experiments suggest that this may indeed be the case when both isotypes recognize the same epitope. Theoretically, low levels of IgA could be blocked or outcompeted by IgM HLA alloantibodies. However, IgA, like IgG, is an antibody isotype that is produced after T cell-dependent germinal center reactions, which causes class switching and affinity maturation. While we cannot formally rule out interference of IgM, we expect this to be as infrequent as with IgG detection. Finally, serum IgA occurs in the monomeric (87%) and polymeric (13%), usually dimeric form.[34] Monomeric IgA has only two antigen-binding sites whereas dimeric IgA has four, which may influence binding the IgA antibody to the beads and further detection of the antibody. However, because of low levels of dimeric IgA in serum, this is unlikely to have an impact on the observed frequency of IgA HLA antibodies.

In this study we used samples from patients with a history of allogeneic HLA exposure. We selected patients from several clinical scenarios to make an inventory of the presence of IgA HLA antibodies in these groups. Although we found no evidence that serum IgA HLA antibodies are involved in ABMR, IgA HLA antibodies may cause a more chronic rejection phenotype through the effect of antibody dependent cell mediated cytotoxicity (ADCC).[35] Therefore, studying the presence of IgA HLA-specific antibodies in patients who are diagnosed with chronic ABMR is an interesting, but yet unexplored area. In addition, we did not detect any IgA HLA antibodies in serum from patients with functioning grafts. Therefore, we cannot support any of the hypotheses about the potential role of IgA HLA antibody in transplantation outcome.

In summary, we have modified and validated a luminex IgA HLA screening assay and used this assay for the detection of HLA-specific antibodies of the IgA isotype. Using this assay, IgA HLA antibodies were detected only at a low frequency among individuals exposed to HLA alloantigens via different routes of sensitization. Screening of a larger number of samples in a systematic way with this modified assay and including clinical outcome parameters may shed more light on the possible role of IgA HLA antibody in transplantation.

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CONFLICT OF INTEREST

The authors declare no potential conflict of interest.

AUTHOR CONTRIBUTIONS

Helena Car: performed experiments, analyzed data, wrote the manuscript. Gonca E. Karahan: designed the experiments, analyzed data, wrote the manuscript. Simone H. Brand-Schaaf: analyzed data. Geertje J. Dreyer and Aiko P. J. de Vries: selected and provided study samples. Cees van Kooten: interpreted results. Cynthia S.M. Kramer: generated HLA-specific recombinant mAbs. Dave L. Roelen: interpreted results. Frans H. J. Claas: designed the study, interpreted results, wrote the manuscript. Sebastiaan Heidt: interpreted results, revised the manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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